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Abstract: Alpine ecosystems are vulnerable to ever-changing environmental conditions, leading to shifts in vegetation distribution and composition with implications on soil functionality and carbon (C) turnover. Although deadwood represents an important global C stock, scarce information is available on how slope exposure influences the wood-inhabiting microbiota throughout the decomposition process in an Alpine setting. We therefore evaluated the impact of slope exposure (north- vs. south-facing sites) on physicochemical and microbiological properties (microbial abundance based on real-time PCR: fungal 18S rRNA, dinitrogen reductase [nifH]; microbial biomass: double strand DNA; and microbial activity: hydrolytic enzyme activities of the main nutrient cycles) of Picea abies wood blocks and the underlying soil in a field experiment in the Italian Alps during a three-year period. Overall, a higher abundance of fungi and nitrogen-fixing bacteria was recorded in the soil at the north-facing site where cooler and moister conditions were observed. In contrast, no exposure effects were found for these two microbial groups in the wood blocks, while their abundance increased over time, accompanied by more acidic conditions with progressing decay. The impact of exposure was also enzyme specific and time dependent for both the P. abies wood blocks and the underlying soil.

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Chemical and microbiological changes in Norway spruce deadwood during the early stage of decomposition as a function of exposure in an Alpine setting

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Abstract

Alpine ecosystems are vulnerable to ever changing environmental conditions, leading to shifts in vegetation distribution and composition with implications on soil functionality and carbon (C) turnover. Although deadwood represents an important global C stock, scarce information is available on how slope exposure influences the wood-inhabiting microbiota over the decomposition process in an alpine setting. We therefore evaluated the impact of slope exposure (north- *vs.* south-facing sites) on physico-chemical and microbiological properties (microbial abundance based on real-time PCR: fungal 18S rRNA, dinitrogen reductase (*nifH);* microbial biomass: double strand DNA; and microbial activity: hydrolytic enzyme activities of the main nutrient cycles) of *Picea abies* wood blocks and the underlying soil in a field experiment in the Italian Alps over a 3-year period. Overall, a higher abundance of fungi and nitrogen-fixing bacteria was recorded in the soil at the north-facing site where cooler and moister conditions were observed. In contrast, no exposure-effects were found for these two microbial groups in the wood blocks, while their abundance increased over time, accompanied by more acidic conditions with progressing decay. The impact of exposure was also enzyme-specific and time-dependent for both the *P. abies* wood blocks and the underlying soil.

1. Introduction

Mountain alpine ecosystems have gained increasing attention over the past years because they are particularly sensitive to ever changing climatic conditions (Egli et al., 2006, 2009). Indeed, an increase of about 2 °C has been recorded in the annual minimum temperature in the European Alps during the 20th century (Beniston et al., 1997). As a consequence, previous studies have reported the occurrence of an upward migration of tree and shrub species in alpine environments owing to rising temperatures (Motta and Nola, 2001; Dullinger et al., 2003). Furthermore, changes in land-use and management also constitute a dominant factor affecting soil biodiversity in Alpine ecosystems (Meyer et al., 2013). Altogether this can ultimately entail changes in the quality and quantity of soil organic matter (SOM), as well as in the vegetation composition and/or activities of soil organisms (Myers et al., 2001; Djukic et al., 2010; Siles et al., 2017), with implications for both carbon (C) dynamics and soil functionality (Theurillat and Guisan, 2001; Allison et al., 2010; A'Bear et al., 2014).

Among the organic C reservoirs, deadwood represents a global C-store estimated to be in the range of 73 ± 6 Pg (Pan et al., 2011). This is mainly due to its high lignin content resulting in a slow C turnover rate making its decomposition dynamics determinant for the soil C balance (Floudas et al., 2012; Moroni et al., 2015). As woody material decays, its physico-chemical properties gradually change over time, which can lead to a succession of microbial communities in wood according to their biochemical requirements and nutrient availability (Rajala et al., 2012). Fungi (brown rot-, soft rot-, and white rot-) are considered the main wood decomposers, having different wood-degradation strategies. In particular, white-rot fungi are capable to degrade all components of the wood cell wall (including lignin) by secreting a plethora of extracellular lignocellulolytic enzymes (van der Wal et al., 2007; Kielak et al., 2016). There exists also evidence of complex fungal-bacterial interactions, both positive and negative, within the deadwood environment as reviewed by Johnston et al. (2016), even though, the identity and ecology of bacterial communities in decomposing wood remained underexplored compared to fungi. For instance, one assumes that fungi might be able to meet the

nitrogen requirements for their vegetative and generative growth through the associations with nitrogen-fixing bacteria. Accordingly, Hoppe et al. (2014) found positive correlations between fungal sporocarps and the richness of *nifH* (dinitrogen reductase) genes in deadwood logs from *Fagus sylvatica* and *Picea abies*. This is also in line with recent findings from Gómez-Brandón et al. (2017) who observed that fungal abundance (qPCR-based) was strongly correlated with *nifH* abundance in *P. abies* coarse woody debris at different stages of natural decay.

Several factors were found to act as drivers for the changes in wood-inhabiting microbiota such as the soil type (Sun et al., 2013), wood physico-chemical properties, particularly density, pH, moisture content, total lignin and cellulose (Purahong et al., 2014; Hoppe et al., 2016). The wood decay stage, with an increase in fungal and bacterial abundance as wood decomposes (Gómez-Brandón et al., 2017), together with the host tree species (Hoppe et al., 2016) are also of importance. In addition, topographic features, particularly the slope exposure, may influence the deadwood decay dynamics in subalpine environments (Petrillo et al., 2015; Fravolini et al., 2016). However, there is still a paucity of information about how slope exposure and climate, in general, affect deadwood inhabiting microbiota in alpine environments (Gómez-Brandón et al., 2017).

Therefore, we performed a field mesocosm experiment with equally sized wood blocks of *Picea abies* to evaluate the effect of exposure (north- *vs.* south-facing slope) on the abundance of nitrogen-fixing bacteria (*nifH* gene) and fungi assessed by real-time PCR. In addition, several potential enzymatic activities that are involved in the main nutrient cycles were determined during the early stage of decomposition (0 – 156 weeks) of deadwood in an alpine setting. Furthermore, we assessed which physico-chemical parameters were the most important drivers shaping the microbial communities in wood and in the underlying soil.

We hypothesised that: 1) the microbial biomass and activity will be more favoured at the south- than at the north-facing slope during *P. abies* wood decomposition in a high alpine setting and that such exposure-effects will be time-dependent; 2) the changes in fungal and *nifH* gene

abundances as a function of exposure will be more evident at the end of the monitoring owing to an increased nutrient availability as woody decay progresses.

2. Material and Methods

2.1. Experimental set-up

Two study sites were selected at an altitude of 2400 m above sea level (a.s.l.) in Val di Rabbi (Trentino, Italy) on a north- (N_5) and south-facing (S_{10}) slope, respectively. We selected natural grasslands close to the border of the potential tree line in order to minimise the influence of human activities and the grazing by livestock, and to assess how such sites might be affected by the input of wood as trees advance in elevation. Both alpine sites belong to an already well-known climosequence (Egli et al., 2006; Fravolini et al., 2016; Bardelli et al., 2017) and they were located in catchments with acidic paragneiss (Bardelli et al., 2017). The soils were classified as Podzol (north-facing site) and Cambisols (south-facing site; Egli et al., 2006), and are sandy to silty (N_5: sand 53%, silt 28%, clay 19%; S_{10}: sand 51%, silt 27%, clay 21%) according to Bardelli et al. (2017). Mean annual precipitation is around 1300 mm, mean annual air temperature about -1.0°C (Sboarina and Cescatti, 2004) and mean annual soil temperature ranges from 2.2 °C to 4.5 °C (Egli et al., 2016) at north- and south-exposure, respectively.

A field experiment using open mesocosms was set up at both study sites with the purpose of monitoring the early stage of *P. abies* (L.) Karst deadwood decomposition as a function of slope exposure and time in i) wood blocks and ii) the topsoil layer (0–5 cm) that is in intimate contact with the wood blocks. Mesocosms (PVC tubes, 10.2 cm and 25.0 cm diameter and height, respectively) were installed into the natural soil in summer 2012, prior to the addition of the wood blocks of *P. abies* at a distance of > 0.5 m from the adjacent mesocosms, leaving at the surface a border of about 1 cm. From the mesocosm set-up (August 2012) to the placement of the wood blocks (June 2013) one year passed in order to permit normal conditions to be restored and as such, the decay monitoring study would be performed under undisturbed conditions. Considering that the

size and geometry of deadwood may have a strong influence on the decay processes (Van der Wal et al., 2007), equal-sized (5 cm × 5 cm × 2 cm) wood blocks of *P. abies* were directly placed on the soil surface, in each of the mesocosm tubes. Three replicate mesocosms for each time point were installed in each of the study sites. The wood blocks and the topsoil layer (0–5 cm) were collected (using lab-gloves) in July 2014 (t1; 52 weeks), in July 2015 (t2; 104 weeks) and in July 2016 (t3; 156 weeks); resulting in a total of 18 samples for each substrate (= 2 sites × 3 times × 3 replicates), with three wood blocks kept as controls (t0). Prior to the placement of the wood blocks into the mesocosms, five soil sub-samples (t0) were collected in the surrounding area of the mesocosm. All samples were kept in cooling boxes and transported to the laboratory. The soil samples were sieved (< 2 mm), the wood blocks were air-dried at room temperature, cut-milled (4 mm; Retsch mill) and aliquoted into 50-ml sterile conical centrifuge tubes. The samples were then stored at 4 °C and -20 °C for physico-chemical and (micro)biological analyses, respectively.

2.2. Wood and soil physico-chemical analyses

The fresh and dry weight of the wood blocks were determined to assess the wood mass as a function of progressing decay as described in Fravolini et al. (2016) and Petrillo et al. (2016). Wood (1 g fresh weight, fw) and soil (5 g, fw) samples were oven-dried (105 °C) for 24 h to determine their dry weight. The volatile solid (VS) concentration was determined from the weight loss following ignition in a muffle furnace (Carbolite, CWF 1000) at 550 °C for 5 h. Total C and nitrogen contents of the oven-dried samples were analysed using a CN analyser (TruSpec CHN; LECO, Michigan, U.S.A.). Electrical conductivity (EC) and pH were determined in water extracts (1:10 and 1:20, w/v for soil and wood, respectively) using a conductivity Meter LF 330 (WTW,Weilheim, Germany) and a pH meter Metrohm 744, respectively. Inorganic nitrogen (NH_4^+, NO_3^-) was measured in 0.0125 M $CaCl_2$ soil extracts (Kandeler, 1993 a,b). Both the total and available P contents in soil samples were determined according to the ascorbic acid method (Kuo, 1996).

160 *2.3. Potential enzyme activities*

161 A heteromolecular exchange procedure by using a 4% solution of lysozyme as desorbant and a
162 bead-beating agent (Fornasier and Margon, 2007) was performed to assess the following hydrolases
163 in wood (0.1 g, fw) and soil (0.2 g, fw) samples: i) C-cycle: β-glucosidase (*gluc*), xylosidase (*xylo*),
164 cellulase (*cell*); ii) N-cycle: chitinase (*chit*), leucine-aminopeptidase (*leu*); iii) P-cycle: alkaline and
165 acid phosphomonoesterases (*alk*P and *ac*P). In order to perform the enzymatic multiple assay for
166 wood extracts, some modifications were required as described by Gómez-Brandón et al. (2017). All
167 measurements were performed in duplicate for each field replicate, and the activities were
168 expressed as nanomoles of 4-methyl-umbelliferyl (MUF) min^{-1} g^{-1} dry (wood/soil).

169

170 *2.4. Molecular analyses*

171 *2.4.1. Wood and soil microbial biomass index (dsDNA)*

172 Direct extraction of total wood (0.1 g, fw) and soil (0.2 g, fw) DNA followed by PicoGreen-based
173 quantification of *crude* (not purified) double stranded DNA (dsDNA) was performed to estimate
174 wood and soil microbial biomass (Fornasier et al., 2014). Some modifications were required to
175 determine the dsDNA content in wood as described in Gómez-Brandón et al. (2017).

176

177 *2.4.2. DNA extraction*

178 The whole community DNA was extracted from wood (0.1 g, fw) and soil samples (0.2 g, fw) and
179 purified by using a commercial kit (FastDNA Kit for Soil, MP-Biomedicals) as described in Ascher
180 et al. (2009) for further downstream analyses. For wood samples, one ceramic sphere (Lysing
181 Matrix E, MP, Biomedicals) was added to the lysing tubes, so as to guarantee for an accurate cell
182 disruption of the woody tissue. DNA extracts were qualitatively and quantitatively characterised as
183 described in Bardelli et al. (2017).

184

185 *2.4.3. Quantitative real-time PCR*

186 Quantitative real-time PCR (qPCR) was performed to determine the 18S rRNA gene copy number
187 of fungi, the abundance of the functional gene *nifH* in the wood and the soil samples. qPCR was
188 carried out using a Rotorgene 6000 Real Time Thermal Cycler (Corbett Research, Sydney,
189 Australia) in combination with the Rotorgene Series Software 1.7. To build the standard curves we
190 used the purified PCR products of known concentrations of the following pure cultures as
191 templates: *Fusarium solani* (DSMZ 10696) – fungi; *Azospirillum irakense* (DSMZ 11586) – *nifH*
192 gene. The primer pairs were: FF390/FR1 (fungi; Prévost-Bouré et al., 2011); and nifHf/nifHr (*nifH*
193 gene; Töwe et al., 2010). Stock concentration [gene copies μL^{-1}] was determined via PicoGreen
194 measurement and freshly prepared for each run. Ten-fold dilutions ranging from 10^9 to 10^2 copies
195 μL^{-1} were used for the standard curve construction. Quantitative PCR was performed in 20-µL
196 volumes. Each reaction mix contained 1X Sensimix™ SYBR® Hi-rox (Bioline, USA), forward and
197 reverse primers (200 nM each primer), 0.4 mg mL^{-1} BSA, distilled water (RNase/DNase free,
198 Gibco™, UK) and 2 µL of either 1:10 diluted DNA-extract, and ten-fold diluted standard DNA. All
199 the standards and samples were run in duplicate. After an initial denaturation at 95 °C for 10 min,
200 thermal cycling comprised 40 cycles of 15 s at 95 °C, 30 s at 50 °C, 30 s at 72 °C for fungi; and 45
201 s at 95 ºC, 45 s at 55 ºC, 45 s at 72 ºC for *nifH* gene. To check for product specificity and potential
202 primer dimer formation, runs were completed with a melting analysis starting from 65 °C to 95 °C
203 with temperature increments of 0.25 °C (0.5 °C for *nifH* gene) and a transition rate of 5 s. The
204 purity of the amplicons was checked by the presence of a single band of the expected length on a
205 1% agarose gel stained with the DNA stain Midori Green (Nippon Genetics, Germany) and
206 visualised by UV-transillumination (Vilber Lourmat Deutschland GmbH).
207
208 *2.5. Statistical analyses*
209 A factorial analysis of variance (ANOVA) was done to evaluate the effect of exposure (north *vs.*
210 south exposure) and time (t0, t1, t2, t3) on wood and soil physico-chemical and microbiological
211 parameters. Normality and variance homogeneity of the data were tested prior to ANOVA using the

212 Shapiro-Wilks´ and Levene´s tests, respectively. Prior to analysis, data were log- or square root-
213 transformed to meet the assumptions for ANOVA (when it was necessary). Significant differences
214 ($p \leq 0.05$) were analysed by paired comparisons with the Tukey HSD test. A non-parametric test
215 (Kruskal-Wallis test) was performed when the data did not meet the normality condition.
216 Associations between the potential enzymatic activities and the main chemical and microbiological
217 variables were explored by Pearson's correlation. These analyses were performed using Statistica 9
218 (StatSoft, USA). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance was
219 performed using R 3.1.2 (Open Source Software) to map the wood and soil physico-chemical
220 parameters to the shifts in microbial biomass (dsDNA), microbial abundance (qPCR) and hydrolytic
221 enzyme activities as a function of slope exposure and time. The lengths of the arrows indicate the
222 direction of maximum correlation of the physico-chemical parameters and the significance level
223 was assessed with a permutation test implemented in the envfit function of vegan library (Oksanen
224 et al., 2008).
225
226 **3. Results**
227 An overview of the wood physico-chemical and (micro)biological parameters as a function of
228 exposure (N- *vs.* S-facing slope) and time (0 – 156 weeks) is given in Tables 1 and 3 and those for
229 the soil in Tables 2 and 3. The statistical results are shown in Table 4.
230
231 *3.1. Wood physico-chemical parameters*
232 Exposure had a significant impact on the wood moisture content, with higher values at the N- than
233 at the S-facing slope (Tables 1 and 4). Moreover, a 7-fold increase in moisture levels was recorded
234 after 52 weeks compared to the beginning of the experiment at both slopes, followed by a decrease
235 until the end of the monitoring period (156 weeks). The wood VS concentration was not
236 significantly influenced by the slope exposure, and neither exposure nor time significantly affected
237 the wood mass (Tables 1 and 4). In addition, the lowest pH value was measured at the end of the

monitoring period (156 weeks) at both the N- and the S-facing slopes (Tables 1 and 4). The exposure-effect on EC of the wood was only evident after 156 weeks, with higher values (3 times higher) at the S- than at the N-facing slope. A significant decrease in EC was found after 52 weeks (3 – 5 times lower than at t0), followed by an increase until the end of the monitoring at both slopes (Tables 1 and 4). Exposure did not have a significant impact on the wood total C content (Tables 1 and 4). The nitrogen content in the wood blocks was below the detection limit irrespective of the exposure and time.

3.2. Soil physico-chemical parameters

Soil moisture levels were significantly higher at the N- than at the S-facing slope, and the lowest values were found after 104 weeks at both slopes (Tables 2 and 4). The VS concentration was almost twice as high at the N- than at the S-facing site, whereas no significant changes were found over time (Tables 2 and 4). Slope exposure had a significant impact on the soil pH after 52 weeks, with an average value of 5.0 at the N- and 5.5 at the S-facing site (Tables 2 and 4). A significant decrease in soil pH was recorded after 52 weeks at the N-facing slope, but it remained stable afterwards until the end of the monitoring period. No significant differences in soil pH with time were observed at the S-facing slope (Tables 2 and 4). The soil EC was at the S-facing site twice as high as at the N-facing site at the beginning and at the end of the monitoring period (156 weeks); while no exposure-effects were found at the intermediate sampling times. Additionally, the highest EC value was recorded after 156 weeks at both slopes (Tables 2 and 4). In general, the N-facing slope showed higher total C and nitrogen contents compared to the S-facing one, regardless of the duration of the experiment (Tables 2 and 4). There was no a significant exposure-effect on the NH_4^+ and NO_3^- contents. Both parameters, however, significantly varied over time. Indeed, a very distinct increase in NH_4^+ was measured after 52 weeks at both slopes, followed by a continuous increase (2 times) at the end of the monitoring period at the N-facing slope (Tables 2 and 4). Concerning the NO_3^- content, the lowest values were generally detected after 104 and 156 weeks at both N- and S-

facing slopes (Tables 2 and 4). The total P content was neither influenced by slope exposure nor by time (Tables 2 and 4). Overall, higher available P levels were observed at the S- than at the N-facing slope irrespective of time, except for the last sampling point (no exposure-effect). Moreover, the highest available P levels were recorded after 104 weeks at both slopes, followed by a significant decrease after 156 weeks at the S-facing slope (Tables 2 and 4).

3.3. Wood (micro)biological parameters

Wood microbial biomass assessed as double stranded DNA yield (dsDNA) was up to 3-times higher at the S- than at the N-facing slope irrespective of time (Tables 3 and 4). Moreover, a significant increase in the dsDNA content was found over time at both slopes (Table 3). Fungal and *nifH* gene abundances in wood were not significantly affected by the slope exposure; however, significant differences were recorded in both microbial groups over time (Tables 3 and 4). The highest *nifH* abundance was recorded after 104 and 156 weeks at the N- and the S-facing slope, respectively. A reduction in the fungal abundance was registered after 52 weeks at both slopes, followed by an increase after 104 weeks reaching similar values to those observed in the initial wood blocks (Table 3). β-glucosidase, xylosidase and cellulase activities did not vary significantly in terms of exposure (Table 4); however, a sharp increase (around 16-times higher for β-glucosidase and 60-fold higher for xylosidase and cellulase activities) was observed after 104 weeks compared to the beginning of the experiment at both slopes (Figs. 1A–C). Afterwards, a decrease in these three enzyme activities was recorded after 156 weeks at the N-facing slope. The exposure-effect on the chitinase, leucine-aminopeptidase and alkaline phosphomonoesterase activities was time-dependent (Figs. 2A-C; Table 4). Higher activities (between 4- and 6-times higher) were recorded at the S- than at the N-facing slope after 104 and 156 weeks; while no exposure-effect was found after 52 weeks (Figs. 2A-C). Moreover, the highest activity of the three aforementioned enzymes was registered at the end of the monitoring at both slopes. Although the acid phosphomonoesterase

activity was not significantly influenced by slope exposure (Table 4), a pronounced increase was found over time compared to the beginning of the monitoring period (Fig. 2D).

3.4. Soil (micro)biological properties

Soil microbial biomass (dsDNA) was neither influenced by slope exposure nor by time (Tables 3 and 4). Nonetheless, slope exposure had a significant impact on soil fungal and *nifH* gene abundances (Table 4), being approximately 3-times higher at the N- than at the S-facing slope irrespective of the time (Table 3). Overall, the β-glucosidase, xylosidase and cellulase activities were 2-times higher at the N- than at the S-facing slope after 104 weeks (Figs. 1D–F); whereas no exposure-effect was observed for the other sampling times, except for the xylosidase activity at the beginning of the monitoring period (N > S; Fig. 1E). However, no significant differences over time were recorded for these enzyme activities (Figs. 1D–F; Table 4), except for the β-glucosidase at the S-facing site (52 > 104 weeks; 2-times higher). Chitinase activity significantly varied in terms of exposure only after 156 weeks (S > N; Fig. 2E, Table 4), while no significant differences were detected over time irrespective of the slope exposure (Table 4). The exposure-effect on the leucine-aminopeptidase and alkaline phosphomonoesterase activities was also time-dependent (Table 4). A higher activity was recorded at the S- than at the N-facing slope after 52 weeks; whereas the opposite trend (N > S) was found after 104 weeks (Fig. 2F & G). Furthermore, both enzymes showed higher activities at the last two sampling times (104 and 156 weeks) at the N-facing site; while a higher activity was recorded after 52 weeks at the S-facing one. Nevertheless, acid phosphomonoesterase activity was significantly higher at the N- than at the S-facing slope regardless of the sampling time (Fig. 2H; Table 4).

3.5. Non-metric multidimensional scaling (NMDS) analysis

Overall, the *P. abies* wood blocks collected at the beginning of the monitoring (t0) clustered in the negative side of the first ordination axis (Fig. 3A), indicating that they represent a specific

315 microhabitat. All the other wood samples were located in the positive side of the first axis (Fig. 3A),
316 being wood pH (R^2 = 0.45, $p \leq 0.01$) the major determinant for this differentiation. Additionally,
317 along the second axis a separation as a function of exposure was mainly observed for the wood
318 samples collected at the end of the monitoring period (i.e., 156 weeks; t3). Wood moisture appeared
319 to be the most determinant factor for this differentiation (R^2 = 0.31, p = 0.06). Furthermore, a clear
320 separation in terms of slope exposure was recorded for the soil samples collected at the N- and the
321 S-facing slopes (negative and positive sides, respectively) along the first ordination axis (Fig. 3B).
322 The major physico-chemical parameters responsible for this differentiation were total C (R^2 = 0.51,
323 $p \leq 0.001$), total nitrogen (R^2 = 0.40, $p \leq 0.01$) and available P (R^2 = 0.40, $p \leq 0.01$), followed by
324 VS (R^2 = 0.38, $p \leq 0.05$) and soil moisture (R^2 = 0.35, $p \leq 0.05$).
325
326 **4. Discussion**
327 In line with previous studies performed in the Trentino area (Egli et al., 2006, 2009;
328 Fravolini et al., 2016; Bardelli et al., 2017), the soils located at the N-facing slope were moister and
329 richer in total C and nitrogen contents compared to those at the S-facing slope. Along a
330 climosequence, Fravolini et al. (2016) observed faster decay rates of the *Picea abies* wood blocks at
331 N- than at S-facing slopes up to an elevation of 1800 m a.s.l., probably due to the higher soil
332 moisture and clay content along with a lower soil pH. These are favourable conditions especially for
333 fungi which can better develop on N-facing sites. Nevertheless, we did not observe distinct changes
334 in the *P. abies* wood blocks mass over the three-year observation period. In general, this fits well
335 with findings of Fravolini et al (2016) and Petrillo et al. (2016) who measured very slow and
336 sometimes almost barely measureable decay rates of *Picea abies* in alpine environments with wood
337 mass decay rates often between 0.018 to 0.040 y^{-1}. These authors explained the low decay rates to
338 be due to the climatic conditions and the extremely slow decomposition rate of lignin.
339 In this study, the higher soil moisture and OM levels recorded at the northern slope were
340 accompanied by higher soil fungal and *nifH* gene abundances in comparison with the southern

slope. Bardelli et al. (2017) did not observe an exposure-effect on soil fungal communities (qPCR-based) up to an altitude of 1800 m a.s.l. in the same study area. These discrepancies in terms of exposure might be related to the higher proportion of grassland and a colder climate registered at 2400 m a.s.l. compared to the other study sites surveyed by Bardelli et al. (2017). Indeed, complex interactions between local scale factors, such as soil properties and vegetation composition are known to largely affect soil microbial communities and their spatial distribution in mountain environments (Ascher et al., 2012; Regan et al., 2017; Siles et al., 2017).

In contrast to soil, exposure did not have a significant impact on the two previously mentioned microbial groups (fungi and *nifH*) in the *Picea abies* wood blocks. This is in disagreement with our second hypothesis. However, a general increase in their microbial abundance was observed over time. This phenomenon could be ascribed to the release of nutrients with progressing wood decay, which provide a source of nutrients for new colonising microorganisms – microbial succession (Hoppe et al., 2015). Moreover, more acidic conditions (lower pH values) were detected in the *P. abies* wood blocks at the end of the monitoring period that might lead to favourable conditions for the fungal growth and their activity. In accordance with Baldrian et al. (2016) and Gómez-Brandón et al. (2017), we also found that pH was one of the most influential driving factors shaping the wood microbial abundance and activity during the early stages of decomposition. Hoppe et al. (2015) reported, however, that variations in the abundances of bacterial phyla (using pyrosequencing analysis) are rather determined by a combination of several wood properties (i.e., C and nitrogen contents, wood moisture) than by single parameters such as pH alone.

Although previous studies pointed to an existing association between fungi and diazotrophic bacteria in wood (Hoppe et al., 2014; Johnston et al., 2016; Gómez-Brandón et al., 2017), we did not find a significant interaction between these two microbial groups in terms of abundance. This might be due to the fact that we focused on early stages of decomposition of Norway spruce deadwood. Indeed, Hoppe et al. (2014) found a stronger positive correlation between fungal

fructification and *nifH* diversity during the intermediate stage of decay of *P. abies* and *Fagus sylvatica* logs. Another plausible explanation could be related to the moisture content, since it is known to be one of the limiting factors of the biological nitrogen fixation (Rinne et al., 2017). In fact, a decrease in wood moisture level was found at the end of the monitoring period in our study.

Furthermore, enzyme-specific exposure effects were observed for the *P. abies* wood blocks as previously shown by Gómez-Brandón et al. (2017). On the one hand, the C-related enzyme activities were not affected in terms of exposure, while, on the other hand, those activities involved in the N-cycle (leucine-aminopeptidase and chitinase) and P-cycle (alkaline phosphomonoesterase) showed higher activities at the southern slope. This thermal signal (S > N) was time-dependent (i.e., after 104 and 156 weeks), partially corroborating our first hypothesis. This is in line with the fact that the wood samples at the S-facing slope were characterised by a higher microbial biomass (based on dsDNA). Accordingly, the dsDNA yields were positively correlated with most of the previously mentioned enzymes (*data not shown*) at the end of the monitoring period, probably due to more suitable conditions (i.e., higher nutrient availability) for microbial growth as wood decay progresses (Gonzalez-Polo et al., 2013; Petrillo et al., 2015, 2016; Pastorelli et al., 2017). In fact, the increase in fungal abundance after 104 weeks was also accompanied by an increase in the C-related enzyme activities in the *P. abies* wood blocks, suggesting the higher activity of the wood-inhabiting fungi at this stage of decay.

As occurred with wood the exposure-effect on soil enzyme activities related to C- and N-cycles was also time-dependent. Nonetheless, soil microbial biomass (dsDNA) did not vary in terms of exposure which is in agreement with Bardelli et al. (2017). These authors, however, found higher levels of microbial biomass on northern slopes by using the substrate-induced respiration approach. The different effect of exposure on soil microbial biomass could be interpreted in the light of the "method-result effect" (Nannipieri et al., 2003; 2017). Furthermore, we observed a higher soil acid phosphomonoesterase activity at the northern slope over time probably due to the lower soil P

availability at this slope than in the comparable S-facing one. Indeed, an increase in P acquiring enzyme activities is expected under conditions of soil P deficiency (Fraser et al., 2015).

5. Conclusion

Our field mesocosm experiment enabled us to observe how different thermal and moisture conditions (due to exposure effects) affected the wood and soil inhabiting microbiota in terms of their abundance and activity in the studied alpine setting. A higher abundance of fungi and nitrogen fixing bacteria was recorded in the topsoil layer at the N-facing site characterised by cooler and moister conditions. In the *P. abies* wood blocks, however, the abundance of these two microbial groups did not respond to exposure while an increase in their abundance was observed with progressing wood decay along with more acidic conditions. This points to wood pH as a crucial driving factor of the deadwood microbiota during the early decay stages. The impact of exposure was also enzyme-specific and time-dependent for both the *P. abies* wood blocks and the underlying soil. Altogether, this indicates the importance of using multiple methods that include a broad array of enzymes to avoid potential misinterpretation of effects on specific nutrient cycles (Nannipieri et al., 2012) caused by environmental changes in Alpine ecosystems.

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578

579 **Figure captions**

580 **Figure 1.** Potential activities of β-glucosidase (A & D), xylosidase (B & E) and cellulase (C & F) of
581 the wood and soil samples collected in August 2013 (0 weeks), July 2014 (52 weeks), July 2015
582 (104 weeks), and in July 2016 (156 weeks) at the north- and the south-facing sites (N_5 and S_{10},
583 respectively). Values are means (n = 3) with standard deviation.
584

585 **Figure 2.** Potential activities of chitinase (A & E), leucine aminopeptidase (B & F), alkaline
586 phosphomonoesterase (C & G) and acid phosphomonoesterase (D & H) of the wood and soil
587 samples collected in August 2013 (0 weeks), July 2014 (52 weeks), July 2015 (104 weeks), and in
588 July 2016 (156 weeks) at the north- and the south-facing sites (N_5 and S_{10}, respectively). Values are
589 means (n = 3) with standard deviation.
590

591 **Figure 3.** Non-metric multidimensional scaling (NMDS) ordination was performed to map the
592 physico-chemical parameters to the shifts in wood (A) and soil (B) microbiological properties
593 (microbial abundances and enzyme activities) as a function of time (t0 = control, July 2014; t1 =

after 52 weeks, July 2015; t2 = after 104 weeks, July 2016; t3 = after 156 weeks) and slope exposure (N-facing slope = point symbol; S-facing slope = triangle symbol).

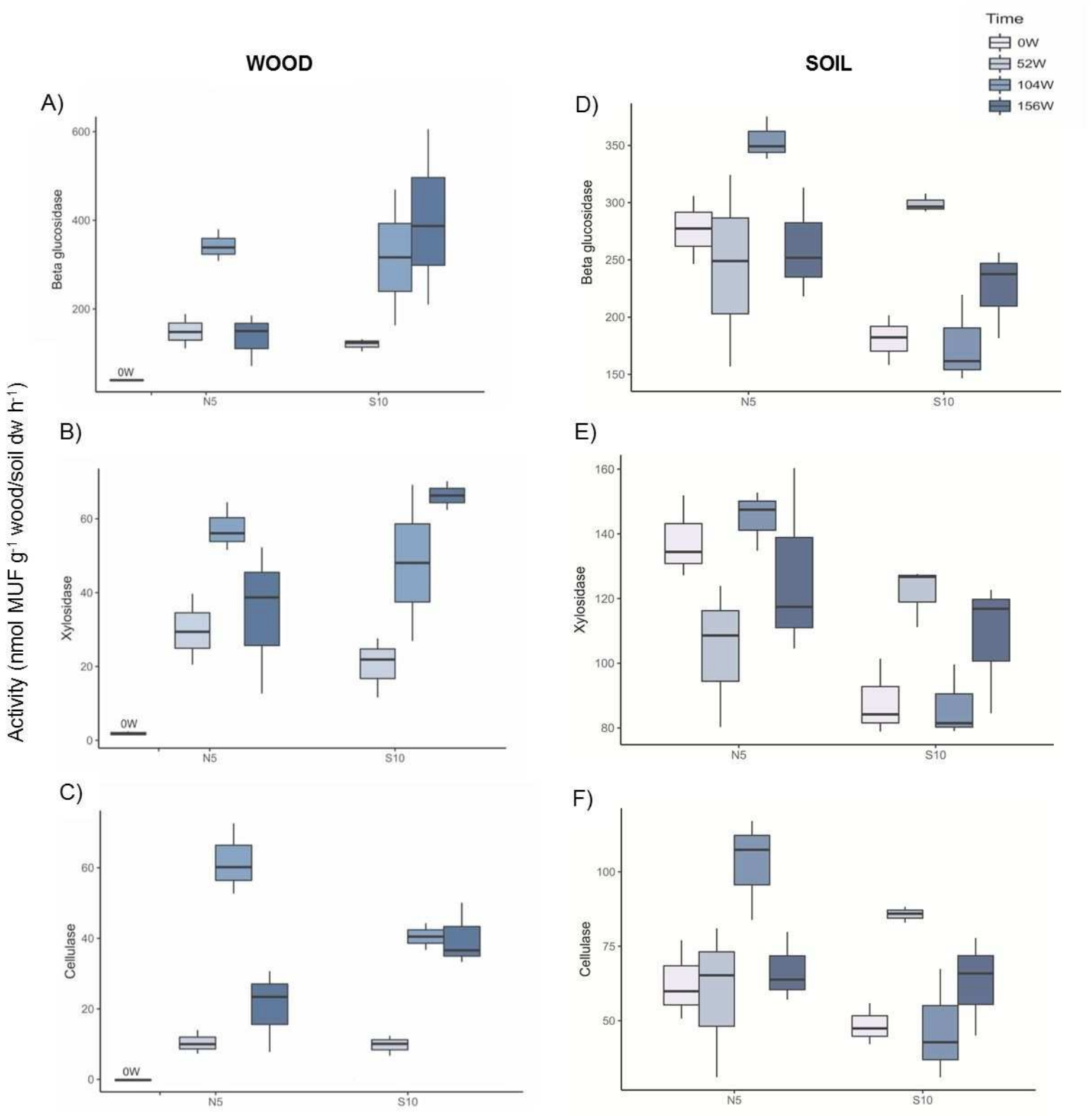

WOOD
SOIL
Time
0W
52W
104W
156W
A)
B)
C)
D)
E)
F)
Beta glucosidase
Xylosidase
Cellulase
Activity (nmol MUF g-1 wood/soil dw h-1)
0W
N5
S10

Fig. 1

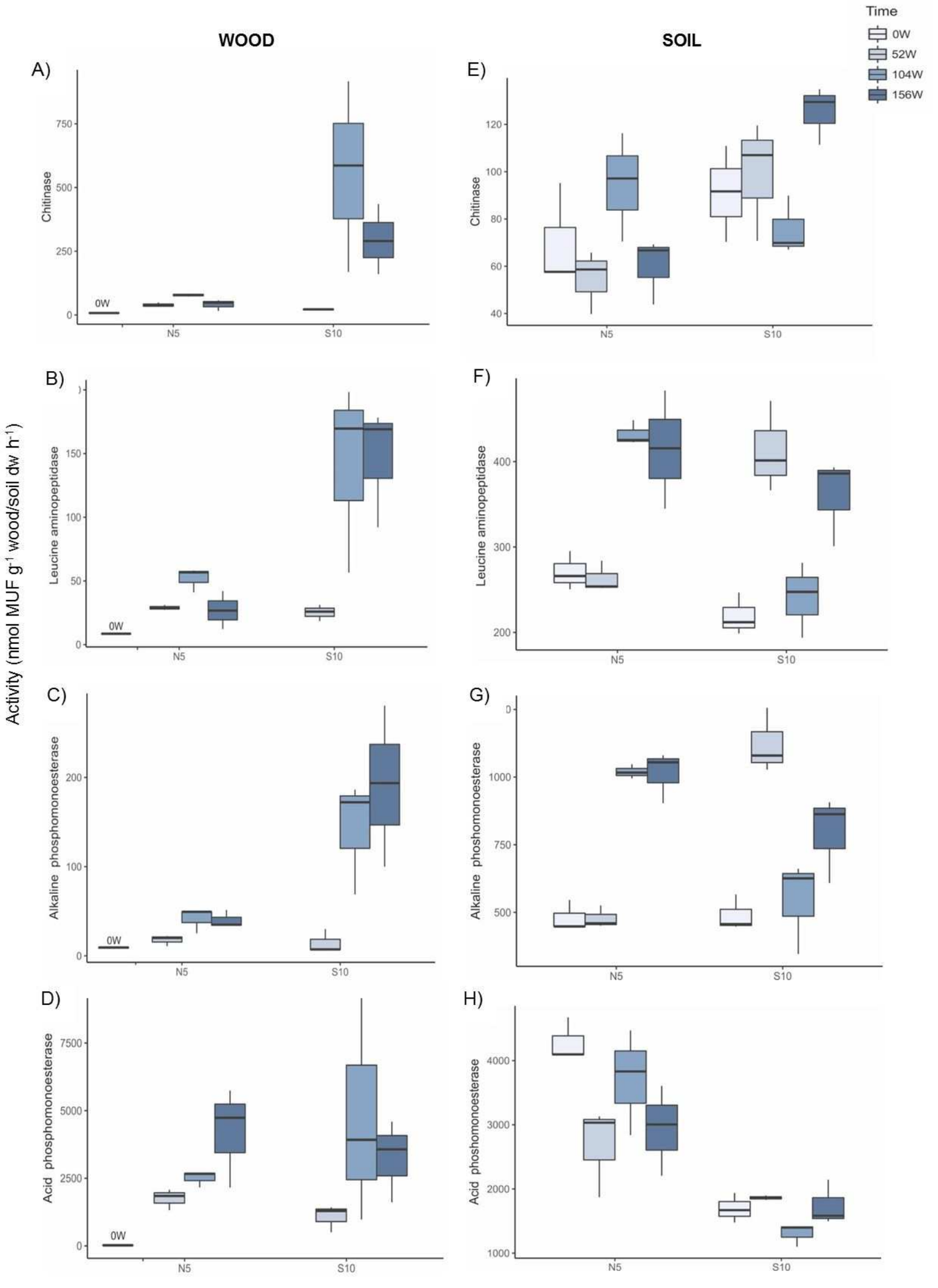
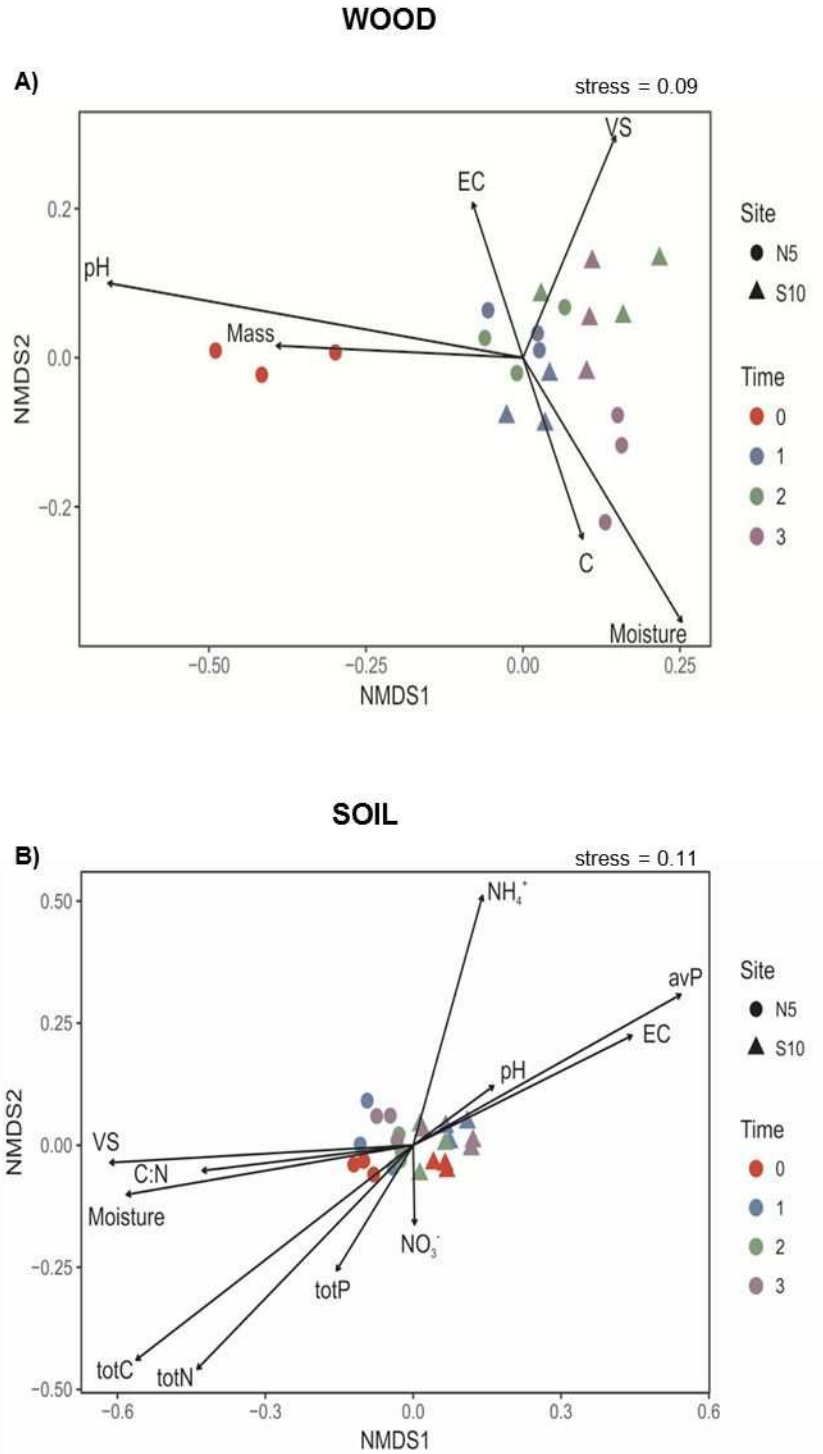

WOOD
SOIL
Time
0W
52W
104W
156W
A)
B)
C)
D)
E)
F)
G)
H)
Chitinase
Leucine aminopeptidase
Alkaline phosphomonoesterase
Acid phosphomonoesterase
Alkaline phoshomonoesterase
Acid phoshomonoesterase
Activity (nmol MUF g-1 wood/soil dw h-1)
N5
S10
0W

Fig. 2


WOOD
A)
stress = 0.09
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EC
pH
Mass
C
Moisture
NMDS1
NMDS2
Site
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Time
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SOIL
B)
stress = 0.11
NH4+
avP
EC
pH
VS
C:N
Moisture
NO3-
totP
totC
totN
NMDS1
NMDS2
Site
N5
S10
Time
0
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2
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Fig. 3

Table 1.

Physico-chemical properties of the wood samples collected in August 2013 (t0), July 2014 (t1; 52 weeks), July 2015 (t2; 104 weeks), and in July 2016 (t3; 156 weeks) in the *in-field* mesocosm experiment at north- and south-facing sites (N_5 and S_{10}, respectively) located at the same elevation (2400 m a.s.l.). Values are means (n=3) with standard deviations in brackets. Data are expressed on a dry weight (dw) basis.

Sites	**Time**	**Moisture** (%)	**Volatile solids** (%)	**Wood mass** (g)	**pH**	**Electrical Conductivity** ($\mu S\ cm^{-1}$)	**Total C** (%)
	t0	8.2 (2.0)	99.2 (0.8)	22.5 (0.0)	5.5 (0.1)	51.7 (6.5)	49.0 (0.3)
N_5	t1	54.2 (2.8)	99.0 (0.5)	21.1 (1.1)	5.4 (0.1)	9.8 (1.4)	50.7 (1.3)
	t2	29.4 (7.9)	99.8 (0.2)	24.3 (2.0)	5.3 (0.1)	32.4 (8.7)	50.5 (1.0)
	t3	39.1 (17.1)	99.6 (0.2)	16.8 (6.2)	5.1 (0.1)	28.5 (6.9)	51.2 (0.9)
S_{10}	t1	56.1 (4.8)	99.0 (0.2)	23.1 (0.9)	5.2 (0.1)	19.1 (10.1)	55.0 (8.0)
	t2	21.1 (7.9)	99.9 (0.2)	19.2 (0.8)	5.1 (0.1)	29.4 (10.2)	51.3 (0.9)
	t3	17.6 (4.5)	99.5 (0.1)	20.4 (3.1)	4.9 (0.1)	77.3 (21.6)	50.4 (0.5)

Table 2.

Physico-chemical properties of the soil samples collected in August 2013 (t0), July 2014 (t1; 52 weeks), July 2015 (t2; 104 weeks), and in July 2016 (t3; 156 weeks) in the *in-field* mesocosm experiment at north- and south-facing sites (N_5 and S_{10}, respectively) located at the same elevation (2400 m a.s.l.). Values are means (n=3) with the standard deviations in brackets. Data are expressed on a dry weight (dw) basis.

Sites	Time	Moisture (%)	Volatile solids (%)	pH	Electrical Conductivity ($\mu S\ cm^{-1}$)	Total C (%)	Total N (%)	NH_4^+ ($mg\ kg^{-1}$ dw)	NO_3^- ($mg\ kg^{-1}$ dw)	Total P ($mg\ kg^{-1}$ dw)	Available P ($mg\ kg^{-1}$ dw)
N_5	t0	54.9 (0.4)	41.1 (1.0)	5.5 (0.1)	15.8 (0.8)	23.5 (1.4)	1.4 (0.1)	4.3 (0.6)	10.2 (3.1)	802.9 (346.3)	4.0 (0.8)
	t1	52.5 (4.0)	40.6 (8.1)	5.0 (0.2)	19.2 (4.1)	20.7 (3.7)	1.1 (0.2)	54.6 (8.0)	15.6 (2.3)	821.6 (552.1)	9.3 (3.1)
	t2	40.1 (6.7)	43.0 (5.1)	5.0 (0.1)	38.9 (4.2)	23.5 (2.0)	1.3 (0.1)	52.0 (15.6)	6.1 (1.9)	891.6 (111.9)	33.0 (7.0)
	t3	51.5 (4.3)	41.8 (7.5)	5.2 (0.01)	39.3 (14.1)	21.9 (5.2)	1.2 (0.3)	117.7 (55.1)	5.6 (2.2)	870.6 (319.6)	25.3 (14.1)
S_{10}	t0	37.5 (4.4)	22.7 (12.0)	5.3 (0.01)	29.4 (0.3)	17.0 (1.4)	1.0 (0.01)	3.3 (0.8)	14.9 (8.9)	931.9 (128.2)	21.3 (8.4)
	t1	49.1 (3.2)	33.0 (4.1)	5.5 (0.1)	26.9 (3.1)	16.3 (2.6)	1.1 (0.2)	97.2 (10.5)	10.4 (1.6)	611.2 (482.9)	46.1 (8.6)
	t2	30.2 (10.2)	26.2 (1.0)	5.3 (0.1)	37.7 (4.5)	13.5 (2.0)	0.9 (0.1)	62.4 (25.7)	6.0 (2.8)	722.9 (312.6)	61.6 (3.1)
	t3	35.4 (1.9)	26.1 (1.7)	5.3 (0.1)	60.5 (10.5)	12.0 (1.5)	0.8 (0.2)	84.3 (16.0)	9.1 (1.3)	450.8 (264.0)	30.0 (3.5)

Table 3.

Microbiological properties of the wood and soil samples collected in August 2013 (t0), July 2014 (t1; 52 weeks), July 2015 (t2; 104 weeks), and in July 2016 (t3; 156 weeks) in the *in-field* mesocosm experiment at north- and south-facing sites (N_5 and S_{10}, respectively) located at the same elevation (2400 m a.s.l.). Values are means (n=3) with the standard deviations in brackets. Data are expressed on a dry weight (dw) basis.

	Sites	**Time**	**Microbial biomass index** (µg dsDNA g^{-1})	**Fungi** (gene copy number g^{-1})	***nif*H gene** (gene copy number g^{-1})
WOOD		t0	1.4 (0.2)	3.12×10^9 (1.67×10^9)	8.77×10^6 (1.37×10^6)
	N_5	t1	9.0 (0.9)	1.66×10^9 (3.87×10^8)	1.46×10^7 (3.57×10^6)
		t2	10.5 (4.3)	3.20×10^9 (1.20×10^9)	3.75×10^7 (2.44×10^7)
		t3	11.6 (4.5)	3.83×10^8 (1.88×10^8)	2.32×10^7 (9.73×10^6)
	S_{10}	t1	12.6 (4.3)	9.24×10^8 (3.30×10^8)	1.51×10^7 (1.90×10^6)
		t2	30.0 (9.9)	2.98×10^9 (4.24×10^8)	2.61×10^7 (6.58×10^6)
		t3	26.5 (17.2)	2.26×10^9 (1.29×10^9)	4.06×10^7 (3.15×10^7)
SOIL	N_5	t0	120.7 (11.3)	1.96×10^9 (2.91×10^8)	1.47×10^8 (1.91×10^7)
		t1	124.2 (5.4)	1.06×10^9 (2.40×10^8)	1.74×10^8 (7.97×10^7)
		t2	128.4 (4.2)	1.88×10^9 (5.39×10^8)	1.10×10^8 (3.13×10^7)
		t3	140.1 (19.5)	1.45×10^9 (2.06×10^8)	1.74×10^8 (3.91×10^7)
	S_{10}	t0	127.3 (12.4)	4.38×10^8 (2.72×10^8)	3.57×10^7 (5.29×10^6)
		t1	128.1 (15.8)	5.83×10^8 (2.83×10^8)	3.90×10^7 (7.68×10^6)
		t2	102.1 (3.7)	5.99×10^8 (2.56×10^8)	5.69×10^7 (1.75×10^7)
		t3	123.7 (18.1)	5.47×10^8 (7.39×10^8)	5.04×10^7 (2.21×10^7)

Table 4.

Factorial ANOVA of the soil and wood physico-chemical and microbiological parameters as a function of slope exposure and time at the N- and S-facing sites (N_5 and S_{10}, respectively) located at the same elevation (2400 m a.s.l.).

	Exposure		**Time**		**Exposure × Time**	
SOIL	F	p	F	p	F	p
Moisture	**30.34**	***	**9.60**	***	2.31	ns
Volatile solids	**32.67**	***	0.63	ns	0.88	ns
pH	**12.43**	**	**6.51**	**	**9.47**	***
EC	**20.38**	***	**23.59**	***	**3.27**	*
Total C	**46.17**	***	1.40	ns	1.44	ns
Total N	**17.71**	***	1.87	ns	1.99	ns
NH_4^+	0.05	ns	**41.68**	***	3.12	ns
NO_3^-	0.32	ns	**6.26**	**	1.77	ns
Total P	1.41	ns	0.43	ns	0.64	ns
Available P	**54.24**	***	**22.83**	***	**5.53**	**
dsDNA	2.38	ns	1.76	ns	2.34	ns
Fungi	**42.21**	***	1.43	ns	1.99	ns
*nif*H	**86.20**	***	0.54	ns	1.95	ns
gluc	**13.77**	**	1.32	ns	**8.28**	**
xylo	**15.34**	**	0.11	ns	**5.99**	**
cell	3.45	ns	1.89	ns	**7.18**	**
chit	**13.64**	**	0.80	ns	**5.72**	**
leu	**4.70**	*	**12.24**	***	**17.08**	***
*ac*P	**85.00**	***	1.70	ns	**4.71**	*
*alk*P	0.04	ns	**13.50**	***	**21.05**	***
WOOD	F	p	F	p	F	p
Moisture	**4.93**	*	**44.27**	***	2.31	ns
Volatile solids	0.04	ns	**12.16**	**	na	na
Mass	0.01	ns	2.75	ns	na	na
pH	**14.20**	**	**36.57**	***	1.87	ns
EC	**9.04**	**	**26.39**	***	**4.54**	*
Total C	0.01	ns	**9.60**	*	na	na
dsDNA	**12.13**	**	**21.49**	***	2.64	ns
Fungi	0.27	ns	**5.64**	**	1.71	ns
*nif*H	0.13	ns	**11.15**	***	0.65	ns
gluc	2.30	ns	**18.15**	***	1.54	ns
xylo	1.09	ns	**44.17**	***	1.47	ns
cell	1.08	ns	**19.92**	***	0.42	ns
chit	**12.96**	**	**45.92**	***	**9.60**	***
leu	**16.67**	***	**43.52**	***	**8.56**	**
*ac*P	0.03	ns	**19.75**	***	0.82	ns
*alk*P	**9.07**	**	**35.74**	***	**5.99**	**

pH (pH H_2O), EC (Electrical conductivity), NH_4^+ (Ammonium content), NO_3^- (Nitrate content), dsDNA (double stranded DNA; soil and wood microbial biomass index), Fungi (18S rRNA gene copy number), *nifH* (Bacterial *nif*H gene copy number), *gluc* (β glucosidase), *xylo* (xylosidase), *cell* (cellulase), *chit* (chitinase), *leu* (leucine-aminopeptidase), *ac*P (acid phosphomonoesterase), *alk*P (alkaline phosphomonoesterase).

na (not available); ns (not significant); * p <0.05; ** p <0.01; *** p <0.001